PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A01N 37/18, A61K 38/00	A1	(11) International Publication Number: WO 00/38515
		(43) International Publication Date: 6 July 2000 (06.07.00)

(21) International Application Number: PCT/US98/27633

(22) International Filing Date: 24 December 1998 (24.12.98)

(71) Applicant *(for all designated States except US)*: NEW YORK MEDICAL COLLEGE [US/US]; Valhalla, NY 10595 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: FERRONE, Saldono [-/US]; - (**).

(74) Agent: GALLAGHER, Thomas, C.; ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: PEPTIDE MIMICS USEFUL FOR TREATING DISEASE

(57) Abstract

The present invention provides a method of treating disease, such as melanoma, in a mammal comprising treating the mammal with an effective amount of a peptide mimic that elicits an immune response against a target molecule associated with a disease.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

PEPTIDE MIMICS USEFUL FOR TREATING DISEASE

BACKGROUND OF THE INVENTION

Current immunotherapeutic approaches to treating disease, such as melanoma, rely on the passive administration of large amounts of antibodies to angiogenesis targets such as the HMV-MAA and tumor associated antigen. Limitations of this therapeutic strategy include the difficulty of administering large amounts of monoclonal antibodies to a patient and maintaining a constant high level of antibodies over a long period of time in a patient.

In recent years there has been growing interest in the development and application of active specific immunotherapy of malignant melanoma as well as all cancer and disease in general. This trend reflects, at least in part, the lack of effective therapy once the disease has metastasized and the rather disappointing results of clinical trials of antibody based passive immunotherapy. Among the various types of available immunogens, peptides derived from human melanoma associated antigen which express T cell defined epitopes are most popular in the scientific community. The interest in this approach reflects i) the significant progress made in recent years in our understanding of the molecular steps which lead to the development of an immune response and to the recognition and destruction of target cells by CTL and ii) the identification of peptides utilized by CTL to recognize melanoma cells. The validity of immunotherapy with peptides, which is being evaluated in a number of clinical trials in progress, is indicated by clinical responses observed in about 40% of the immunized patients. However, the success of this therapeutic strategy which relies exclusively on T cell mediated destruction of melanoma cells is likely to be negatively affected by structural and/or functional abnormalities of HLA Class I antigens and/or antigen processing machinery, which are frequently found in melanoma lesions and especially in metastases. The latter finding is of particular concern, since at present most of the trials of T cell-based immunotherapy enroll only patients with metastases.

Because of the potential negative impact of abnormalities in HLA Class I antigen and/or antigen processing machinery on the outcome of active specific immunotherapy, which relies exclusively on T cell immunity, there is need to induce both humoral and cellular immunity to the HMW-MAA.

Although not immunogenic in patients with melanoma, the HMW-MAA has been selected for active specific immunotherapy, since it is expressed in a high percentage of melanoma lesions with limited heterogeneity, it has a restricted distribution in normal tissues, its recognition is expressed in the immune system of patients with melanoma and it plays a role in the metastatic potential of melanoma cells. Therefore that anti-HMW-MAA antibodies will have a beneficial effect on the clinical course of the disease not only by mediating immune destruction of melanoma cells, but also by interfering with the function of the HMW-MAA in the biology of melanoma cells.

SUMMARY OF THE INVENTION

The present invention provides a method of treating disease, such as melanoma, in a mammal comprising treating the mammal with an effective amount of a peptide mimic that elicits an immune response against a target molecule associated with a disease.

The present invention also provides a peptide mimic that elicits an immune response against a target molecule.

The invention also provides a method of treating a disease, in a mammal comprising treating the mammal with an effective amount of a vector that expresses a peptide mimic that elicits an immune response against a target molecule associated with a disease.

The invention further provides a nucleic acid molecule that encodes a

peptide mimic that elicits an immune response against a target molecule associated with a disease.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a method of treating disease, such as melanoma, in a mammal. The method comprises treating the mammal with an effective amount of a peptide mimic that elicits an immune response against a target molecule associated with the disease. The disease may be cancer, arthritis, macular degeneration, psoriasis, ischemia or any other pathological condition. The animal is preferably a mammal, which may be a human or an animal typically used for experimentation, such as mice, rats or rabbits, or a farm animal, such as a cow, horse or pig.

In addition to providing the methods of the invention, the present invention also provides the peptide mimics used in these methods. The peptide mimics of the invention induce an effective immune response when properly presented to the immune system. The immune response preferably inhibits, i.e. prevents, slows or stops, disease such as tumor growth, and therefore inhibits or eliminates the pathological condition associated with the disease.

The peptide mimics of the invention may mimic any target molecule associated with the process of disease, such as, but not limited to, high molecular weight melanoma associated antigen. Further, the peptide mimic may mimic receptors associated with the process of disease, for example, flk-1, flt-1, and KDR; or integrins such as the vitronectin receptor $\alpha_v\beta_3$; or vascular endothelial cadherins (VE-Cadherin-1 and VE-Cadherin-2); TIE-1, TIE-2/Tek, EGFr, PDGF, Her-2, Her-4, Flt-4. However, these are examples of molecules and receptors, and any target molecule involved in disease may be utilized in the invention. A target molecule is defined as a molecule that is present in a mammal during a disease state. The target molecule may be, but need not be, expressed aberrantly during the disease state. The target molecule can be, but

does not need to be limited to, a glycosylated or unglycosylated polypeptide; or a glycolipid, such as gd3.

The peptide mimic may be obtained from a phage library or may be synthetically derived. The peptide mimic may relate to a fragment of an antigen, epitope or antigenic determinant.

The HMW-MAA and the GD_3 ganglioside are useful targets for immunotherapy, but any target molecule associated with disease may be used in the invention. Furthermore, each antigen is independently expressed in at least 60% of melanoma lesions. Therefore, their combined use is expected to minimize the negative impact of antigenic heterogeneity of melanoma cells on the outcome of immunotherapy of melanoma. Lastly, immunity to these two melanoma associated antigens (MAA) appears to affect melanoma cells through different functional and immune-mediated mechanisms and, therefore, is expected to have an additive detrimental effect on melanoma cells.

There are beneficial effects of humoral anti-HMW-MAA immunity elicited with anti-idiotypic (anti-id) mAb on the clinical course of malignant melanoma. These findings corroborate the validity of active specific immunotherapy with mimics of MAA. However, immunotherapy with anti-id mAb which bear the internal image of HMW-MAA and GD_3 ganglioside suffers from the following major limitations: i) inability to generate HLA Class I restricted, HMW-MAA specific CTL, ii) inability to induce a T cell dependent anti-GD_3 ganglioside immune response and iii) low reactivity of anti-anti-id antibodies with the original antigen. Therefore, using i) peptide mimics of HMW-MAA can induce anti-HMW-MAA antibodies as well as HLA Class I restricted, HMW-MAA specific CTL, ii) peptide mimics of GD_3 ganglioside can induce a T cell dependent anti-GD_3 ganglioside immune response and iii) the immune response to self-HMW-MAA and GD_3 ganglioside triggered by the corresponding peptide mimics can be markedly enhanced by boosting hosts with the original antigen.

Characterization of the immunogenicity of HMW-MAA and of GD_3 ganglioside peptide mimics as well as of the enhancement of the immune response to a self-MAA by boosting hosts with the original antigen will contribute to develop novel strategies for active specific immunotherapy in patients with melanoma.

The invention provides active specific immunotherapy of malignant melanoma by utilizing as immunogens, peptide mimics of the human high molecular weight-melanoma associated antigen HMW-MAA). Although not immunogenic in patients with melanoma, this antigen has been selected for active specific immunotherapy, since it is expressed in at least 80% of melanoma lesions, it has a restricted distribution in normal tissues and its recognition is expressed in the immune repertoire of patients with melanoma.

Peptide mimics of HMW-MAA, or any antigen, are obtained by panning phage display peptide libraries with human and mouse anti-HMW-MAA antibodies. Peptide mimics of HMW-MAA are able to elicit immunity to a self antigen in patients with melanoma, although the original antigen is not. Peptide mimics of HMW-MAA are similar, but not identical to the HMW-MAA. Therefore they stimulate clones which recognize HMW-MAA, but have not been deleted during the establishment of self-identity because of their reduced affinity for the antigen. An example of this approach is the induction of anti-carcinoembryonic antigen (CEA) antibodies in patients immunized with anti-idiotypic (anti-id) monoclonal antibodies (mAb) which mimic CEA, but not in those immunized with CEA. Furthermore, the recent report of a simultaneous humoral and cellular immune response to an antibody defined tumor associated antigen in a patient with melanoma, demonstrates that peptide mimics of HMW-MAA with HLA Class I antigen binding motifs can elicit not only a humoral, but also a cellular anti-HMW-MAA immune response. Therefore, peptide mimics of HMW-MAA are more effective immunogens for immunotherapy of melanoma than anti-id mAb which bear its internal image.

The use of such peptide mimic is not limited to treating melanoma, but is useful in treating any disease for which a target molecule is available. The use of such peptide mimics elicits both humoral and cellular immune responses when properly presented to the immune system of a mammal.

Peptides with a sequence identical to that of HMW-MAA will not be able to break tolerance to the HMW-MAA in patients with melanoma since the T and B cell clones recognizing the HMW-MAA which is a self antigen have been deleted during the establishment of self-identity. On the contrary, because of their similarity, but not identity with HMW-MAA, peptide mimics of the HMW-MAA identified using phage display peptide libraries are able to stimulate clones which recognize the HMW-MAA, but have not been deleted during the establishment of self-identity because of their reduced affinity. An example of breaking tolerance is the ability of anti-id mAb which mimic CEA to break tolerance to CEA in patients with colorectal carcinoma, while CEA cannot. Furthermore, peptide mimics of HMW-MAA are more effective to implement active specific immunotherapy in patients with melanoma than anti-id mAb which bear its internal image, since i) they may induce HLA Class I restricted, HMW-MAA specific CTL in addition to anti-HMW-MAA antibodies, ii) they will facilitate the development of immunogens resulting from the fusion of peptides with cytokines which will display an enhanced immunogenicity and iii) they will greatly facilitate the development of assays to test immunized patients for development of humoral and especially cellular anti-HMW-MAA immunity.

By panning phage display peptide libraries with human and mouse anti-HMW-MAA antibodies, we have identified peptide mimics of HMW-MAA. The peptides, have binding motifs for HLA-B27 antigen. Additional peptide mimics of HMW-MAA are available using our large panel of human and mouse anti-HMW-MAA antibodies. Peptide mimics of HMW-MAA with binding motifs for HLA Class I alleles with a high frequency in patients with melanoma, such as HLA-A2 antigens. Immunogenicity of peptide mimics of

HMW-MAA is tested in mice and rabbits for example. To optimize the immunogenicity of peptide mimics of HMW-MAA e. several variables including the use of multiple antigenic peptides (MAP), since they have been shown in other antigenic systems to increase the immunogenicity of synthetic peptides.

Peptides which are identified with anti-HMW-MAA antibodies can also be recognized by human CD8+ T cells. We first identify the HLA Class I allospecificity to which the identified peptides can bind utilizing the Bioinformatics and molecular analysis software (BIMAS). Then we assess the validity of this prediction by measuring the binding of the identified peptides to the selected HLA Class I allospecificities. Lastly, we test whether the identified peptides can generate in HLA Class I antigen transgenic mice and in vitro, HLA Class I antigen restricted, HMW-MAA specific CTL. We also test, whether that the peptides identified with anti-HMW-MAA antibodies share a T cell defined epitope with HMW-MAA derived peptides in spite of the lack of amino acid sequence homology. This occurrence is supported by the recently described recognition of two structurally different MHC-peptide complexes by a single T-cell receptor. Peptides which mimic the HMW-MAA and are found to generate in vitro and in HLA Class I antigen transgenic mice, HLA Class I restricted, HMW-MAA specific CTL have the ability to elicit humoral and cellular anti-HMW-MAA immunity in patients with melanoma.

This approach has the advantage of combining the induction of a humoral anti-HMW-MAA immune response, which has already been found to be associated with a statistically significant survival prolongation with a T cell anti-HMW-MAA immunity. Therefore, our approach should be less affected by HLA Class I antigen and antigen processing machinery abnormalities frequently found in melanoma lesions.

The invention provides a means for assessing the immunogenicity of peptide mimics of HMW-MAA, identified by panning phage display peptide libraries with human and mouse anti-HMW-MAA antibodies. This information

will contribute to optimize a novel strategy to design immunogens which may elicit both humoral and cellular anti-HMW-MAA immunity. The improved characteristics of the immunogens are expected to enhance the efficacy of active specific immunotherapy of melanoma.

The present invention overcomes these problems by using active specific immunotherapy against target molecules to inhibit disease. Such methods of immunotherapy against such target molecules include modification of immunogens to cause an immune response against the molecules. Modification of the target antigen can be achieved by, for example, conjugation of immunogenic reagents to the antigen (see US Patent Nos. 5,334,379); haptenization of the antigen (see U.S. Patent Nos. 4,778,752 and 5,290,551); the use of adjuvants bound to, or administered with, the target antigen; binding peptide fragments to the antigen; binding the target antigen to MHC class I and class II restricted antigens (see U.S. Patent No. 4,478,823); and changing glycosylation patterns of the target antigens (see U.S. Patent No. 5,484,735), among other methods.

Another method where immunity is induced to a target molecule is that of a peptide that mimics the target antigen. Since the mimicry of the target "self" antigen by the peptide mimic is likely to be imperfect, such peptide mimics will break tolerance to a self antigen, although administration of the self antigen is not able to do so. It has been previously shown that anti-idiotypes that bear the internal image of carcinoembryonic antigen (CEA) can induce anti-CEA antibodies in patients with colorectal carcinoma, while CEA itself cannot do so. Therefore, an alternative approach to using the actual "self" angiogenesis target antigen as a vaccine is to use either an anti-idiotypic antibody that mimics the antigen, bears an internal image of the antigen, and elicits an immune response. However, the use of anti-idiotypic antibodies is not perfect.

The theory of idiotypic relationships and networks is based on the Jerne model (Jerne, N.K. (1974) Ann. Immunol. (Paris) 125C: 373; Jerne, N.K. et al.

(1982) EMBO 1:234). Thus immunization with an antibody expressing a paratope (antigen combining site) for a given antigen, should result in anti-antibodies (anti-idiotypes), some of which share with the antigen a complementary structure to the paratope. Those anti-idiotypes could then possibly act as antigens, i.e., mimic the antigen. Thus, the immune system would carry within it an internal image of the antigen, the anti-idiotype.

The peptide mimic used in the invention may be a small molecule not native to the mammal or a nucleic acid molecule not native to the mammal. Such small molecules or nucleic acids may be synthesized or isolated from an organism other than the mammal. The immunogen of the invention may also be a peptide molecule, a pseudopeptide or peptidomimetic, capable of eliciting an immune response against a molecules involved in disease. Such peptide mimics may be synthesized or isolated from an organism other than the mammal. Methods for screening small molecules, nucleic acid molecules, peptide molecules and peptidomimetics are well-known in the art. (See, for example, J. Biomolecular Screening, 1 (1), pg 27-31, 1996.)

The peptide mimics of the invention are modified in various ways known in the art, such as by conjugating or genetically fusing the peptide mimics to immunogenic reagent. Conjugation or fusion of the peptide mimics to an immunogenic reagent can stimulate an immune response to the peptide mimics. The conjugates and fused molecules of this invention can be prepared by any of the known methods for coupling or fusing antigens to carriers or fusion molecules. The preferred method of conjugation is covalent coupling whereby the antigen is bound directly to the immunogenic reagent. Preferred immunogenic reagents include polysaccharides (U.S. Patent No. 5,623,057), and peptidoglycans (U.S. Patent No. 5,153,173). These U.S. Patents, as well as all such patents presented in the instant specification, are herein incorporated by reference.

Another method of modifying the peptide mimics of the invention is for them to be bound or genetically fused with a cytokine, lymphokine, hormone or

growth factor (U.S. Patent No. 5,334,379). Examples of such molecules include, but are not limited to, interferons, GM-CSF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6 and IL-7 (U.S. Patent No. 5,334,379). As stated above, these U.S. Patents are herein incorporated by reference.

Another method of modifying the peptide mimic of the invention is haptenization (chemically linking) of the peptide mimic. A hapten is a substance having the ability to, when coupled with a protein, elicit an immune response. The peptide mimic of the invention can itself be haptenized , or can be bound to hapten-modified proteins. (U.S. Patent Nos. 4,778,752 and 5,290,551).

An additional method of modifying the peptide mimics of the invention is glycosylation of the peptide mimics or glycosylation of the carrier molecules of the peptide mimics (see U.S. Patent Nos. 5,484,735 and 4,629,692).

Furthermore, peptidomimetic compounds or pseudopeptides, i.e., compounds which mimic the activity of peptides, may be used in modification of the immunogens of the invention (U.S. Patent Nos. 5,386,011, 5,153,173 and 4,631,270).

Modification of the peptide mimics of the invention by bonding the peptide mimic with a Major Histocompatibility Complex (MHC) antigen, forming a complex that is also useful in this invention. (U.S. Patent No. 4,478,823). The source of such MHC antigens and the methods of bonding the peptide mimics of the invention to the MHC antigens are detailed in the cited U.S. Patent No. 4,478,823, which is herein incorporated by reference.

Equivalent proteins have equivalent amino acid sequences. An amino acid sequence that is substantially the same as another sequence, but that differs from the other sequence by one or more substitutions, additions and/or deletions, is considered to be an equivalent sequence. Preferably, less than 25%, more preferably less than 10%, and most preferably less than 5% of the

number of amino acid residues in a sequence are substituted for, added to, or deleted from the proteins of the invention.

For example, it is known to substitute amino acids in a sequence with equivalent amino acids. Groups of amino acids generally considered to be equivalent are:

(a) Ala(A) Ser(S) Thr(T) Pro(P) Gly(G);
(b) Asn(N) Asp(D) Glu(E) Gln(Q);
(c) His(H) Arg(R) Lys(K);
(d) Met(M) Leu(L) Ile(I) Val(V); and
(e) Phe(F) Tyr(Y) Trp(W).

The peptide mimics and modified peptide mimics of the invention elicit an immune response against a target molecule in an animal. The animal is preferably a mammal, such as a rabbit, rat, or mouse. Preferably, the animal is a human. An immune response means production of antibodies, i.e. humoral, and/or a cell-mediated response, such as a T-cell response including helper and cytotoxic T cell responses.

Nucleic acid molecules encoding the peptide mimics may be introduced into mammalian cells by methods known in the art. Such methods have been described, for example, in Mulligan, et al., U.S. patent 5,674,722. The methods described in Mulligan, et al., U.S. patent 5,674,722 for preparing vectors useful for introducing genes into mammalian cells, particularly endothelial cells, are incorporated herein by reference.

The peptide mimic may be presented to the immune system by a vehicle. For example, the peptide mimic may be present on the surface of an antigen presenting cell or combined with a pharmaceutically acceptable carrier or adjuvant.

Antigen presenting cells are generally eukaryotic cells with major

histocompatibility complex (MHC), preferably Class II, gene products at their cell surface. For the purposes of this specification, antigen presenting cells also include recombinant eukaryotic cells such as peripheral blood cells and recombinant bacterial cells. Some examples of antigen presenting cells as defined by this specification include dendritic cells, macrophages that are preferably MHC Class II positive, monocytes that are preferably MHC Class II positive, and lymphocytes. (Also see U.S. Patent No. 5,597,563).

In one embodiment of the subject invention, the antigen presenting cell is a recombinant eukaryotic cell that expresses exogenous DNA encoding the antigen of the invention. The recombinant eukaryotic cell may be prepared in vivo or in vitro.

Suitable cloning/expression vectors for inserting DNA into eukaryotic cells include well-known derivatives of SV-40, adenovirus, cytomegalovirus (CMV), and retrovirus-derived DNA sequences. Any such vectors, when coupled with vectors derived from a combination of plasmids and phage DNA, i.e. shuttle vectors, allow for the cloning and/or expression of protein coding sequences in both prokaryotic and eukaryotic cells.

Other eukaryotic expression vectors are known in the art, e.g., P.J. Southern and P. Berg, J. Mol. Appl. Genet. 1, 327-341 (1982); S. Subramani et al, Mol. Cell. Biol. 1, 854-864 (1981); R.J. Kaufmann and P.A. Sharp, "Amplification And Expression Of Sequences Cotransfected with A Modular Dihydrofolate Reductase Complementary DNA Gene," J. Mol. Biol. 159, 601-621 (1982); R.J. Kaufmann and P.A. Sharp, Mol. Cell. Biol. 159, 601-664 (1982); S.I. Scahill et al, "Expression and Characterization of the Product of a Human Immune Interferon DNA Gene in Chinese Hamster Ovary Cells," Proc. Natl. Acad. Sci. USA 80, 4654-4659 (1983); G. Urlaub and L.A. Chasin, Proc. Natl. Acad. Sci. USA 77, 4216-4220, (1980).

The peptide mimics of the invention may also be presented to the immune system on the surface of recombinant bacterial cells. A suitable

recombinant bacterial cell is an avirulent strain of Mycobacterium bovis, such as bacille Calmette-Guerin (BCG), or an avirulent strain of Salmonella, such as S. typhimurium. The recombinant bacterial cells may be prepared by cloning DNA comprising the active portion of the antigen in an avirulent strain, as is known in the art; see, for example, Curtiss et al., Vaccine 6, 155-160 (1988) and Galan et al., Gene 94, 29-35 (1990) for preparing recombinant Salmonella and Stover, C.K. et al., Vaccines 91, Cold Spring Harbor Laboratory Press, pp. 393-398 (1991) for preparing recombinant BCG.

Cloning vectors may comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences. Some suitable prokaryotic cloning vectors include plasmids from E. coli, such as colE1, pCR1, pBR322, pMB9, pUC, pKSM, and RP4. Prokaryotic vectors also include derivatives of phage DNA such as M13, fd, and other filamentous single-stranded DNA phages.

Vectors for expressing proteins in bacteria, especially E. coli, are also known. Such vectors include the pK233 (or any of the tac family of plasmids), T7, and lambda P_L. Examples of vectors that express fusion proteins are PATH vectors described by Dieckmann and Tzagoloff in J. Biol. Chem. 260, 1513-1520 (1985). These vectors contain DNA sequences that encode anthranilate synthetase (TrpE) followed by a polylinker at the carboxy terminus. Other expression vector systems are based on beta-galactosidase (pEX); lambda P_L; maltose binding protein (pMAL); glutathione S-transferase (pGST) - see Gene 67, 31 (1988) and Peptide Research 3, 167 (1990).

The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, and

promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters of SV40, and other sequences known to control the expression of genes in prokaryotic or eukaryotic cells and their viruses or combinations thereof.

The peptide mimics of the invention may also be combined with a suitable medium. Suitable media include pharmaceutically acceptable carriers, such as phosphate buffered saline solution, liposomes and emulsions.

The peptide mimics may also be combined with pharmaceutically acceptable adjuvants that may enhance the immune response, such as muramyl peptides, lymphokines, such as interferon, interleukin-1 and interleukin-6, or bacterial adjuvants. The adjuvant may comprise suitable particles onto which the immunogen is adsorbed, such as aluminum oxide particles. These compositions containing adjuvants may be prepared as is known in the art.

An example of a bacterial adjuvant is BCG. When used as an antigen presenting cell as described above, recombinant BCG may additionally act as its own adjuvant. In this case, additional adjuvant may not be needed although one or more additional adjuvants may optionally be present. When used in its natural (non-recombinant) state, BCG acts solely as an adjuvant by being combined with the immunogen or anti-idiotypic antibody, resulting in a form that induces an effective immune response.

The peptide mimic or peptide mimic composition may be administered to a mammal by methods known in the art. Such methods include, for example, intravenous, intraperitoneal, subcutaneous, intramuscular, topical, or intradermal administration.

EXAMPLES

Identification of peptide mimics of the HMW-MAA by panning phage display peptide libraries with human anti-HMW-MAA single chain Fv (scFv) C21 scFv C21 which was isolated by panning a human semi-synthetic phage display scFv library with melanoma cells Colo 38, recognizes the HMW-MAA since it immunoprecipitates the two characteristic components (250 kD and >450 kD) of the HMW-MAA from melanoma cell extracts. Immunoscreening of clones isolated by panning phage display peptide libraries LX-8 and X_{15}, with anti-HMW-MAA scFv C21 following the methodology described by Bonnycastle et al., led to the identification of positive clones only among those isolated from the X_{15} library. The reactivity of the clones with scFv C21 was confirmed by ELISA. Nucleotide sequence analysis of 16 randomly selected positive clones from the X_{15} library by the dideoxy nucleotide chain termination method showed inserts with the amino acid sequence SPSWYCPDCDKRPLV in 14 clones, RPYRYDPLGDLKSRH in 1 clone and EARNWHDFPIHPRTL in one clone. Synthetic peptides corresponding to the sequence SPSWYCPDCDKRPLV (synthetic peptide #1) and EARNWHDFPIHPRTL (synthetic peptide #2) reacted with scFv C21 in a binding assay. However, only synthetic peptide #1 inhibited the binding of scFv C21 to HMW-MAA bearing melanoma cells Colo 38.These results suggest that peptide #1 binds to the antigen combining site of scFv C21, while peptide #2 binds outside the antigen combining site of scFv C21. The reactivity of peptide #1 with scFv C21 is conformation dependent since the peptide linearized with dithiothreitol (to break the disulfide bonds) did not react with scFv C21. Peptide #1 is a mimotope of the determinant recognized by scFv C21 since a comparison of the amino acid sequence of peptide #1 with that of the HMW-MAA did not reveal any homology.

Identification of peptide mimics of the HMW-MAA by panning phage display peptide libraries with mouse anti-HMW-MAA mAb 149.53, 225.28, 763.74 and TP61.5.

Monoclonal Antibodies. Mouse anti-HMW-MAA mAb 149.53, 225.28, 763.74 and TP61.5 react with distinct determinants of the HMW-MAA.

Peptide mimics of HMW-MAA identified by mAb 149.53. Panning of phage display peptide libraries LX-8 and X_{15} with anti-HMW-MAA mAb 149.53 resulted in the enrichment of phage displayed peptides from both libraries. Immunoscreening of clones obtained from the two libraries at the end of the fourth round of panning detected positive phages among those isolated from both libraries. The reactivity of phage displayed peptides with mAb 149.53 was confirmed by ELISA. Nucleotide sequence analysis of 5 randomly selected positive clones from each library showed inserts with the amino acid sequence SCRWVGIDLYCP from the LX-8 library and EELHPPGSRAPSIRK from the X_{15} library. A comparison of these amino acid sequences with the published amino acid sequence of the HMW-MAA showed that the amino acids GSRAP identified by mAb 149.53 from the X_{15} library are homologous to amino acid residues 1846-1850 in the sequence of the HMW-MAA.

Peptide mimics of HMW-MAA identified by mAb 225.28. Panning of phage display peptide libraries LX-8 and X_{15} with anti-HMW-MAA mAb 225.28, followed by immunoscreening of clones obtained at the end of the fourth round of panning, detected positive phages only among those isolated from the X_{15} library. The reactivity of phage displayed peptides with mAb 225.28 was confirmed by ELISA. Nucleotide sequencing of the positive clones is in progress.

Peptide mimics of HMW-MAA identified by mAb 763.74. Panning of phage display peptide libraries LX-8 and X_{15} with anti-HMW-MAA mAb 763.74 resulted in the enrichment of phage displayed peptides from both libraries. Immunoscreening of clones obtained from the two libraries at the end of the fourth round of panning detected positive phages only among those isolated from the LX-8 library. The reactivity of phage displayed peptides with mAb 763.74 was confirmed by ELISA. Nucleotide sequence analysis of 8 randomly selected positive clones from the LX-8 library showed peptide inserts with the amino acid sequence QCTGPNVATNCR in 6 clones, TCNGPSVYMNCL in 1 clone and QCTGPNFATNCR in 1 clone. The latter amino acid sequence thus

differed in only one residue from the most frequently represented amino acid sequence. Thus the consensus sequence identified by the clones is XCXGPX(Hy)XXNCX, where Hy represents a hydrophobic amino acid. To corroborate the reactivity of phage displayed peptides with mAb 763.74, synthetic peptides were tested for reactivity with mAb 763.74 in binding and inhibition assays. In a binding assay, synthetic peptide QCTGPNVATNCR, showed reactivity with mAb 763.74 only in the presence of two N-terminal spacer arms (synthetic peptide # 6). Furthermore the peptide did not react with anti-HMW-MAA mAb 149.53, 225.28, TP41.2 (46) and TP61.5 all of which recognize determinants different from that recognized by mAb 763.74. Synthetic peptide # 3 (QCTGPNVATNCR) inhibited the reactivity of mAb 763.74 with HMW-MAA. The reactivity of peptide #3 (QCTGPNVATNCR) with mAb 763.74 is conformation dependent, since the peptide linearized with dithiothreitol did not react with mAb 763.74. It is noteworthy that synthetic peptide # 5 (QCTGPNFATNCR) did not inhibit the reactivity of mAb 763.74 with the HMW-MAA. Thus, the amino acid V appears to play a critical role in the binding of mAb 763.74 to the HMW-MAA. Although a comparison of the identified peptide sequences and the published amino acid sequence of the HMW-MAA did not reveal any homology, the amino acid residues GP occur repeatedly throughout the sequence of the HMW-MAA. Thus the determinant recognized by mAb 763.74 from the phage display peptide library appears to be a mimotope. The immunogenicity of the peptide QCTGPNVATNCR is currently being tested in mice.

Peptide mimics of HMW-MAA identified by mAb TP61.5. Panning of phage display peptide libraries LX-8 and X_{15} with anti-HMW-MAA mAb TP61.5, followed by immunoscreening of clones obtained at the end of the fourth round of panning, detected positive phages among those isolated from both libraries. The ELISA to confirm the reactivity of the positive clones and nucleotide sequence analysis experiments are in progress.

Immunogenicity in BALB/c mice of the peptide mimic of the determinant defined by anti-HMW-MAA mAb 763.74. BALB/c mice were immunized on

day 0, 14, 28, 42 and 63 subcutaneously with the peptide QCTGPNVATNCR (25 or 50 µg/injection) mixed with GM-CSF (10 g/injection; Immunex Corporation, Seattle, WA) and with incomplete Freund's adjuvant (IFA; Sigma Chemical Co., St. Louis. MO). Mice were bled from the retro-orbital sinus, prior to immunization and on day 21, 35, 49, 56 and 70. Immune sera displayed a higher reactivity with HMW-MAA expressing melanoma cells Colo 38 than with HMW-MAA negative B lymphoid cells L14 in a binding assay. The two doses of peptide tested elicited a similar immune response. Representative results are presented in Fig. 6, which also shows the kinetics of the development of antibodies reacting with melanoma cells. These results suggest, but do not prove that the peptide used induces anti-HMW-MAA antibodies. Analysis of the immune sera by immunoprecipitation and SDS-PAGE is in progress to prove this possibility. gd3

Prediction analysis of plausible HLA Class I antigen binding motifs present in the peptides identified by human and mouse anti-HMW-MAA antibodies, scFv C21 and mAb 149.53 and 763.74. In order to determine if the peptide sequences identified by human and mouse anti-HMW-MAA antibodies, scFv C21 and mAb 149.53 and 763.74 can stimulate cytotoxic T cell responses, the peptides were first analyzed for the presence of motifs binding to HLA Class I allospecificities using the BIMAS.

Peptide mimics of HMW-MAA have been selected as immunogens to implement active specific immunotherapy in patients with melanoma instead of peptides derived from the HMW-MAA amino acid sequence, since peptide mimics of HMW-MAA are expected to be more effective in breaking tolerance to a self antigen. Because of their resemblance, but not identity to the HMW-MAA, peptide mimics are expected to be able to stimulate T and B cell clones which recognize the HMW-MAA but have not been deleted during the establishment of self-identity because of their reduced affinity.

Peptides synthesized on the basis of the amino acid sequence of the peptides identified with human and mouse anti-HMW-MAA antibodies are

immunogenic in mice and rabbits. Mice are utilized since i) they are suitable to screen a large number of peptides and of variables which may influence their immunogenicity, ii) they provide a source of hosts with a defined and standardized genetic background which minimizes the variability in the host's immune response, and iii) they allow the testing of variables, i.e. cytokines, dendritic cells, which cannot be tested in other animal species, since the required reagents and/or methodology are not available yet. However, mice do not express the HMW-MAA. Immunohistochemical staining with anti-human HMW- MAA mAb has shown that this antigen is expressed in normal tissues of rabbits with a distribution similar to that found in man. Therefore, rabbits provide us with a model to investigate whether peptide mimics of HMW-MAA can break tolerance to HMW-MAA.

Immunogenicity in mice.

Immunization strategy. The basic immunization design is as follows: BALB/c mice (10 /group) are immunized subcutaneously on day 0,7 and 28. Peptides are injected at the indicated doses, conjugated or non-conjugated to KLH and mixed with one of the adjuvants to be tested. Alternatively peptide pulsed dendritic cells are injected. Sera will be harvested from the retroorbital sinus before immunization and on day 13, 27, 34 and 41. Additional immunizations and/or bleedings are performed on the basis of the results of the analysis of humoral and cellular anti-HMW-MAA immunity.

Effect of peptide dose, immunization number, carrier and adjuvant on the immune response. These experiments test the effect of the dose of peptide, of the number of immunizations, of the conjugation to a carrier and of various adjuvants on the immunogenicity of peptides.

i) Peptides are administered subcutaneously at the dose of 25-125µg/injection, since in preliminary experiments, doses of 25 and 50 g/injection have been shown to induce an immune response as described in the Preliminary Results section.

ii) Peptides are administered at two weeks intervals, two or three times. If no immune response is detected, additional immunizations are given

at two weeks intervals until an immune response is detected.

iii) The effect of conjugation to a carrier, i.e. KLH, on the immunogenicity of peptides are assessed by comparing the immunogenicity of peptides conjugated to KLH with that of non-conjugated peptides. Peptides are coupled to KLH (Pierce, Rockford, IL) using glutaraldehyde for cross-linking. Briefly, peptides are mixed for 1 h at room temperature with KLH at a 1:1 ratio. Following the addition of 0.25% glutaraldehyde, the solution is stirred for an additional 2 h at room temperature. The reaction is terminated by incubating the mixture for 30 min with 1M glycine. The peptide-KLH conjugate is dialyzed overnight at 4 C in PBS. The conjugate is stored at -20 C.

iv) The effect of adjuvant on the immunogenicity of peptides are assessed by injecting peptides mixed with Freund's adjuvant (complete for priming and incomplete for boosting) in a total volume of 200µl or with 10µg of QS21(Aquila Biopharmaceuticals, Inc., Worcester, MA) in a total volume of 200µl.

v) The immunogenicity of peptides presented by dendritic cells are assessed utilizing dendritic cells prepared from bone marrow as described. Briefly, dendritic cells are generated from bone marrow harvested from hind limbs of syngeneic mice. Cells are cultured for 5 days at 37 C in IMDM medium supplemented with 10% FCS, recombinant murine IL-4 (2000 IU/ml; Pepro Tech Inc., Rocky Hill, NJ) and recombinant murine GM-CSF (200 IU/ml; Pepro Tech Inc.). At the end of the incubation, the phenotype of dendritic cells is verified by FACS analysis. Dendritic cells (1×10^6/ml) are incubated for 2h at 37 C in reduced serum Opti-MEM (Gibco Laboratories, Grand Island, NY) supplemented with peptide (10 g/ml). Following washings with HBSS, dendritic cells (1×10^5/mouse) are injected subcutaneously at the base of the tail. The injection of peptide-pulsed dendritic cells are repeated after one week.

vi) The effect of MAP on the immunogenicity of synthetic peptides are tested by injecting subcutaneously 10 g of MAP consisting of synthetic peptides mimicking the HMW-MAA with or without universal T_H epitopes in Freund's adjuvant.

Humoral immune response elicited by synthetic peptides. The methodology utilized to analyze the humoral immune response has been extensively utilized in our previous investigations. It is briefly described below.

i) The level of anti-peptide antibodies is determined by testing serial two fold dilutions of sequential bleedings from immunized mice with synthetic peptides coated on microtiter plates in a binding assay with ^{125}I-labeled anti-mouse IgG and anti-mouse IgM xenoantibodies. The specificity of the binding is assessed by testing immune sera with unrelated peptides coated on microtiter plates and by testing preimmune sera and sera from mice immunized with an unrelated peptide with peptide mimics of HMW-MAA.

ii) The level of HMW-MAA binding antibodies is measured by testing serial two fold dilutions of sequential bleedings from immunized mice with HMW-MAA bearing cultured human melanoma cells in a binding assay with ^{125}I-labeled anti-mouse IgG and anti-mouse IgM xenoantibodies. The specificity of the binding is assessed by testing immune sera for binding to HMW-MAA negative lymphoid cells and by testing preimmune sera and sera from mice immunized with an unrelated peptide with HMW-MAA bearing cultured human melanoma cells.

The specificity of immune sera for HMW-MAA is assessed by SDS-PAGE analysis of the components they immunoprecipitate from ^{125}I- or ^{35}S-labeled melanoma cells and by sequential immunoprecipitation experiments which show that immune sera cannot immunoprecipitate HMW-MAA from a melanoma cell extract immunodepleted with anti-HMW-MAA mAb. Radiolabeling of cells, solubilization of cells, immunoprecipitation, SDS-PAGE and autoradiography or fluorography is performed utilizing procedures we have extensively utilized (46).

If sera from immunized mice do not immunoprecipitate any components from radiolabeled melanoma cells because of the low titer and/or avidity of the antibodies, sera is tested with HMW-MAA purified from a melanoma cell extract by binding to a microtiter plate coated with F(ab')$_2$ fragments of an anti-HMW-MAA mAb. The binding of antibodies to HMW-

MAA is detected with ^{125}I-labeled anti-mouse IgG Fc xenoantibodies. The specificity of the binding is monitored utilizing plates coated with an unrelated antigen.

Sera containing HMW-MAA binding antibodies is tested for staining of melanoma lesions in the immunoperoxidase reaction to prove that the antibodies react with the antigen *in vivo*.

iii) Cross blocking experiments will be performed to map the antigenic determinants recognized by antibodies present in immune sera. To this end, melanoma cells are incubated with saturating amounts (20µg/well) of F(ab')$_2$ fragments of anti-HMW-MAA mAb. Following washing, immune sera are added to cells and the binding of antibodies is detected using ^{125}I-labeled anti-mouse IgG Fc xenoantibodies. Conversely, immune sera is tested for their ability to inhibit the binding of ^{125}I-labeled anti-HMW-MAA mAb to melanoma cells. The specificity of these assays is assessed using mAb and antisera recognizing unrelated antigens.

T cell mediated immune response elicited by synthetic peptides.

i) Proliferative response of lymphocytes is measured by incubating peripheral lymph node cells harvested from immunized mice in triplicate in the presence of synthetic peptides (ranging between 1µM and 50µM) in 96 well flat bottom tissue culture plates at 37 C in a 5% CO_2 humidified atmosphere. After 4 to 5 days incubation, 0.5 µCi [^{3}H] thymidine is added and after an additional 16 h incubation at 37 C, cells are harvested on a cell harvester. Proliferation is assessed as the amount of incorporated [^{3}H] thymidine into cell DNA, as measured by beta scintillation counting of the harvested samples and is expressed as cpm ± SD.

ii) Delayed type hypersensitivity (DTH) responses tested by challenging immunized mice with irradiated (20Krad) HMW-MAA bearing cultured melanoma cells (5x10^5/100µl) or immunizing peptide (75 µg/100µl or 125µg/100µl) in the right hind footpad. Footpad thickness is measured at time 0, 24, 48 and 72 h after the injection of cells or peptide. Results are expressed as mean ± SD of the increase in thickness obtained in at least five mice. Irradiated (20 Krad) HMW-MAA negative human B lymphoid cells

($5x10^5$/100µl) or an unrelated peptide (75µg/100µl or 125µg/100µl) injected into the left hind footpad are used as specificity controls.

Statistical analysis. Depending on whether the values have or do not have a normal distribution, a two sample statistical procedure such as the two sample t test or the two sample Mann-Whitney test will be used to compare the results of the immunological assays in the groups of mice immunized with the various protocols.

Immunogenicity in rabbits

Immunization strategy. The results obtained in mice is utilized to design the strategy to analyze the immunogenicity of peptide mimics of HMW-MAA in rabbits. Peptides are injected at the indicated doses, conjugated or non-conjugated to KLH and mixed with one of the adjuvants found to be the most effective. Sera will be harvested from the rear marginal ear vein before immunization and on day 13, 27, 34 and 41. Additional immunizations and/or bleedings are performed on the basis of the development of humoral anti-HMW-MAA immunity.

Effect of peptide dose, immunization number, carrier and adjuvant on the immune response. The effect of dose, immunization number, carrier, adjuvant and multiple antigenic peptide on the immunogenicity of synthetic peptides will be analyzed utilizing a methodology similar to that described for the same studies in mice.

Humoral immune response elicited by synthetic peptides. The development of anti-peptide antibodies and of anti-HMW-MAA antibodies and the serological and immunochemical characterization of their fine specificity will be assessed utilizing the methodology described for the characterization of the humoral immune response in mice immunized with peptides.

Statistical analysis. The same statistical methods described for the analysis

of the immune response in mice will be utilized to analyze the immune response in rabbits immunized with the various protocols.

To determine whether the peptide mimics of HMW-MAA can induce HLA Class I antigen restricted, HMW-MAA specific CTL *in vitro* and in mice transgenic for the HLA Class I allospecificity binding the immunizing peptide.

Peptide mimics of HMW-MAA elicit humoral anti-HMW-MAA immunity in mice and rabbits and T cell proliferative anti-HMW-MAA response in mice. Peptide mimics of HMW-MAA also can elicit HLA Class I restricted, HMW-MAA specific CTL. This possibility is supported by our preliminary findings that two of the peptides we have identified have HLA-B27.5 binding motifs as described in the Preliminary Results section, by the recognition of the same antigenic determinant by CTL and antibodies (33,34) and by the development of both humoral and cellular immunity to an antibody defined tumor associated antigen in a patient with melanoma. Furthermore, the CTL will be tested for their ability to recognize HMW-MAA in a HLA Class I antigen restricted fashion. This may occur because a partial amino acid sequence homology between peptide mimics of HMW-MAA and peptides which may be derived from HMW-MAA and may be presented by HLA Class I antigens on target cells as described in the Preliminary Results section. Alternatively, the peptides identified with anti- HMW-MAA mAb and HMW-MAA derived peptides presented by HLA Class I antigens may share a CTL defined determinant because of structural homology.

HLA Class I antigen transgenic mice provide a useful model to determine whether peptide mimics of HMW-MAA can stimulate HLA Class I restricted, HMW-MAA specific CTL *in vivo*. Two of the peptides we have identified by panning with anti-HMW-MAA antibodies have binding motifs for HLA-B27.5 as described. These two peptides are tested for their immunogenicity in HLA-B27.5 transgenic mice.

The strategy we will utilize to test whether peptides identified with anti-HMW-MAA mAb generate HLA Class I restricted, HMW-MAA specific CTL include: i) selection, among the identified peptide mimics of HMW-MAA, of peptides which have binding motifs for a HLA Class I allospecificity; ii) assessment of the predicted binding of the selected peptide to the selected HLA Class I allospecificity; iii) *in vitro* sensitization of peripheral blood lymphocytes of an appropriate HLA phenotype with autologous dendritic cells pulsed with the selected peptide; iv) induction of CTL in mice transgenic for the appropriate HLA Class I allospecificity; v) measure of the generation of CTL by testing their ability to lyse TAP deficient cells loaded with the peptide and HMW-MAA bearing melanoma cells in a HLA Class I antigen restricted fashion.

Identification of HLA Class I allospecificity binding motifs in peptide mimics of HMW-MAA. Peptides isolated by panning phage display peptide libraries with anti-HMW-MAA antibodies will be evaluated for binding to various HLA Class I allospecificities using the Bioinformatics and molecular analysis software (BIMAS).

Assessment of the predicted binding of peptides to HLA Class I allospecificities. The relative binding affinities of peptides to selected HLA Class I allospecificities will be assessed in reconstitution assays using the lymphoblastoid cell line T2 transfected with genes encoding HLA Class I allospecificities. Briefly, the assay is performed by treating HLA Class I antigen transfected T2 lymphoblastoid cells with ice cold citric acid- Na_2HPO_4 buffer (mixture of an equal volume of 0.263M citric acid and 0.123M Na_2HPO_4), pH 3.3, for 90 sec. Cells are then buffered with cold Iscove's modified Dulbecco's medium (IMDM), washed with IMDM and incubated with 100 nM human $_2$-μ (Sigma Chemical Co.), 2 μg of anti-HLA-A2,A28 mAb CR11-351 or of anti-HLA-B27 mAb KS4 (depending on the peptide being tested) and either no peptide (negative control), standard peptide (10 μM) or test peptide (10 M). The standard peptides used for HLA-A*0201 and HLA-B*2705 binding have the sequence FLPSDYFPSV and RRYQKSTEL,

respectively. Following a 4 h incubation at room temperature, cells are washed with PBS containing 1% BSA and incubated with FITC-conjugated goat anti-mouse IgG antibodies for 30 min at 4°C. Following washings, cells are resuspended in PBS-1% BSA containing 0.5% paraformaldehyde and analyzed on a FACscan. Results are expressed as fold increase in fluorescence intensity (FI) which is calculated as : FI sample / FI control.

***In vitro* induction of CTL with peptide pulsed dendritic cells.** Dendritic cells will be prepared as described (60) from peripheral blood mononuclear cells (PBMC) of patients with melanoma or of healthy volunteers with the appropriate HLA phenotype. After Ficoll-Hypaque separation, PBMC (1-3 $x10^8$) are cultured in 75 cm^2 culture flasks for 3h at 37 C. The nonadherent cells are removed, and adherent cells are cultured for 5-7 days under sterile conditions in 20 ml of IMDM medium supplemented with 10% heat inactivated human AB serum, 10 mM HEPES, 100 U/ml penicillin-streptomycin, 0.5 mg/ml amphotericin B and 0.03% L-glutamine. Human recombinant GM-CSF (1000 IU/ml; Pepro Tech Inc.) and human recombinant IL-4 (1000 IU/ml; Pepro Tech Inc.) are added every 2-3 days from day 0. Dendritic cells (1 x 10^6/ml) are pulsed with peptide (1 g/ml)for 2 h at 37 C. Autologous $CD8^+$ T cells are prepared from the non-adherent PBMC by positive selection on biomagnetic separation beads (Dynabeads, Dynal Inc., Lake Success, NY). The purity of the $CD8^+$T cell population which is expected to be greater than 95% will be verified by FACS analysis.

$CD8^+$ T cells (4-5 $x10^6$/well) are incubated at 37 C in 24 well tissue culture plates with peptide pulsed dendritic cells ($1x10^6$) for 7 days and then restimulated after 1 week with peptide pulsed dendritic cells. IL-2 (300 IU/ml, Hoffman La Roche, Nutley, NJ) is added 24 h after each stimulation and every 2-3 days thereafter. CTL are tested for specificity after 7-9 days from the original stimulation and 7 days after the restimulation.

Induction of CTL in HLA Class I antigen transgenic mice. Transgenic mice expressing human HLA B*2705 or a chimeric HLA A*0201/K^d are

employed for *in vivo* CTL generation. The A2/K^d transgenic mice are used because it has been shown that expression of the mouse MHC Class I 3 domain enhances the selection of low-avidity CTL in the thymus. Transgenic mice (3/group) are immunized by subcutaneous injection of peptide-pulsed dendritic cells (1 x 10^5/0.2 ml PBS), on day 0 and 7. Ten days after the second injection, mice are sacrificed and the spleens are harvested for *in vitro* restimulation with peptide in IL-2 containing media (60 IU/ml). Restimulations with either peptide-pulsed dendritic cells or splenocytes will occur weekly to maintain the CTL for functional testing.

Assessment of the generation of CTL specific for the peptide used for the *in vitro* and *in vivo* sensitization. The *in vitro* sensitized and *in vivo* induced $CD8^+$ T cells are tested in cytokine-release and cytotoxicity assays for their ability to recognize peptide-pulsed target cells with the appropriate HLA phenotype. In functional assays, TAP-deficient T2 cells expressing the appropriate HLA Class I restriction element, either due to genotype or due to transfection of the corresponding gene, are used as targets to test peptide-specific reactivity. In assays with mouse CTL from A2/K^d transgenic mice, the T2 cells are transfected with the chimeric HLA-A2/K^d gene to improve sensitivity in assays of CTL restricted by the chimeric HLA Class I specificity (61). For the cytotoxicity assay, target cells are pulsed with 1μM peptide for 1 h at 37 C, washed, and then labeled with $Na_2{}^{51}CrO_4$ (150μCi/10^6 cells) for 1-2 h at 37 C. Cells are then washed and mixed with effector cells (at effector/target ratios ranging between 20:1 and 2.5:1) in a final volume of 0.2 ml RPM1 1640 medium containing 10% human AB serum or FBS in 96 well round bottom plates. After a 4 h incubation at 37 C in a 5% CO_2 atmosphere, 100μl of supernatant from triplicate cultures are collected and counted. Data are expressed as the mean percent specific release of ^{51}Cr from target cells. In the cytokine release assays, stimulator cells (1 x 10^5) and responder cells (1 x10^5) are co-incubated in 96-well plates for 24h. Supernatants are harvested and tested by ELISA for the presence of INF-gamma (Endogen, Woburn, MA).

The antigenic specificity of CTL are assessed using TAP-deficient target cells pulsed with an unrelated peptide. The HLA Class I antigen restriction is assessed with blocking experiments using mAb to the HLA Class I allele used as the restricting element, and with target cells pulsed with the peptide used for induction of CTL, but lacking the HLA Class I restricting element.

Assessment of the generation of HLA Class I antigen restricted, HMW-MAA specific CTL. The *in vitro* sensitized and *in vivo* induced $CD8^+$ T cells will be tested in cytokine-release and cytotoxicity assays for their ability to recognize HMW-MAA bearing melanoma cells with the appropriate HLA phenotype. The specificity of CTL for HMW-MAA will be assessed by utilizing tumor cells which express the HLA Class I restriction element, but do not express HMW-MAA. The HLA Class I restriction will be assessed by performing blocking experiments with mAb to the HLA Class I allele used as a restricting element and using melanoma cells which express HMW-MAA, but do not express the HLA Class I restricting element.

Example 2

Peptide mimics of the antigenic determinant(s) recognized by anti-HMW-MAA mAb.

Identification. Positive clones were isolated from both LX-8 and X_{15} libraries by panning with HMW-MAA binding anti-anti-id mAb GH368, GH464, GH586, GH704, GH786 and GH1115, and from the LX-8 library by panning with anti-HMW-MAA mAb 763.74. The phages displayed peptides with different sequences. Synthetic peptides cor-responding to the phage displayed peptide inserts were tested for binding with the corresponding mAb used for panning. The synthetic cyclized peptide #1 QCTGPNVATNCR identified with mAb 763.74 reacted with mAb 763.74 and inhibited its binding to HMW-MAA and to anti-id mAb MK2-23 in a dose dependent fashion. The reactivity of peptide # 1 with mAb 763.74 is conformation dependent, since it was lost upon linearization of the peptide with dithiothreitol. It is noteworthy that the

residue Val at position 7 plays a crucial role in the reactivity of peptide #1 with mAb 763.74, since the synthetic peptide #3 (QCTGPNFATNCR) did not inhibit the reactivity of mAb 763.74 with HMW-MAA and with anti-id mAb MK2-23. The synthetic peptides #5 (GCIKSHPFVRCP) and #7 (NQLPQYMGPAPAYMR) identified with mAb GH368 and GH786, respectively, inhibited the binding of the corresponding mAb to HMW-MAA.

Amino acid sequence homology with HMW-MAA. The peptides identified by mAb GH368 and GH704 and that identified by mAb GH1151 share the amino acids SHPF and PPTFXS with the residues 1457-1460 and 818-823, respectively, of the published amino acid sequence of the HMW-MAA core protein. The LX-8 peptides identified with mAb GH786 and GH1151 share the motif PFQ with the amino acid residues 1458-1460 of the HMW-MAA core protein. Likewise, the peptides identified with mAb GH368, GH586 and GH704 share the motif HPF among themselves. The sharing of the amino acids PF in these two motifs indicates that these residues may play a key role in the expression of the determinant(s) recognized by the anti-anti-id mAb on the HMW-MAA.

Reactivity patterns with anti-HMW-MAA mAb. The structural relatedness among the phage displayed peptides identified with the panel of mAb was analyzed by testing in binding and inhibition assays with mAb 763.74, with the eight HMW-MAA binding anti-anti-id mAb, and with the three HMW-MAA non-binding anti-anti-id mAb. The phage displayed peptides identified with mAb 763.74 reacted with no anti-anti-id mAb. Conversely, mAb 763.74 reacted with the phage displayed peptide identified with mAb GH368, GH464 and GH704. The anti-anti-id mAb GH368, GH704, GH786 and GH1151 reacted with the phage displayed peptides identified by each of them. Furthermore, mAb GH464 and GH586 reacted with the phage displayed peptides identified with mAb GH368 and GH704. mAb GH586 reacted weakly also with the phage displayed peptides identified with mAb GH786. Some of the latter phage displayed peptides reacted weakly with mAb GH518. The synthetic peptide #5 resembled the same as the corresponding phage displayed

peptide in its reactivity with all the mAb, except mAb 763.74 and mAb GH464. The latter two mAb reacted with the phage displayed peptide, but did not react with the corresponding synthetic peptide. It is our working hypothesis that in addition to the peptide sequence, amino acid residues from the pVIII major coat protein of the phage may contribute to the reactivity with mAb 763.74 and GH464. Experiments are in progress to test this hypothesis.

Binding to HLA-A*0201 and to HLA-B*2705 antigens. Analysis with the Bio-informatics and molecular analysis software (BIMAS) has identified HLA-A*0201, -A*6801 and -B*2705 antigen binding motifs in some of the peptides identified with anti-HMW-MAA mAb.

Anti-HMW-MAA mAb have also identified peptides, which have homology to the HMW-MAA derived peptide LLGFSIVAV. The latter has a HLA-A*0201 antigen binding motif. In order to increase the binding to HLA-A*0201 antigens of mAb identified peptides we plan to introduce mutations at positions 2, 5 and 9.

The validity of the prediction of the binding of the identified peptides to HLA Class I alleles is being tested utilizing the stabilization assay. The peptides YMGPAPAYM and CRVELNHPR identified by mAb GH786 and GH704, respectively, stabilized the expression of HLA-A*0201 and HLA-B*2705 antigens, respectively, on HLA-B*2705 transfected TAP deficient T2 (T2-B*2705) cells. The stabilization is specific, since the HLA-B*2705 and the HLA-A*0201 antigen binding peptides induced no detectable change in the expression of HLA-A*0201 and HLA-B*2705 antigens, respectively, on T2-B*2705 cells. The HLA-B*2705 binding peptide, CRVELNHPR stabilizes HLA-B*2705 expression to an extent similar to that induced by the reference peptide RRYQKSTEL.

Immunogenicity in BALB/c mice. BALB/c mice were immunized with phage displayed peptides isolated with mAb 763.74 (QCTGPNVATNCR), mAb GH368 (GCIKSHPFVRCP), and mAb GH786 (TCRLPFQNVACH) from the

LX-8 peptide library and with mAb GH704 (CRVELNHPRAQIMCR) and mAb GH786 (NQLPQYMGPAPAYMR) from the X_{15} library. Sera from mice immunized with all the phage displayed peptides except those identified with mAb GH368 showed a higher reactivity with HMW-MAA expressing melanoma cells Melur than with HMW-MAA negative B lymphoid cells L14 in a binding assay.

Molecular basis of the differential reactivity of HMW-MAA binding mAb with the identified phage displayed peptides. The diversity of the peptides recognized by the panel of anti-HMW-MAA mAb and their ability to inhibit each other in their binding to melanoma cells, are compatible with the recognition of distinct, although spatially close antigenic determinants on the HMW-MAA.

Comparison of the amino acid sequences of the heavy (V_H) and light (V_L) chain variable regions of the anti-HMW-MAA mAb identified a high degree of homology in the amino acid sequences of V_H and V_L CDR of mAb GH368, GH704 , GH786 and GH1151. These four mAb display a very similar reactivity pattern with the phage displayed peptides identified so far. The reduced reactivity of mAb GH586 with the phage displayed peptides isolated with anti-anti-id mAb GH368, GH704 and GH786 is associated with differences in two amino acid residues in V_H-CDR2. Furthermore, the lack of reactivity of mAb GH149 with phage displayed peptides identified with mAb GH368, GH704, GH786 and GH1151 is associated with the replacement of Tyr with Ala in its V_H-CDR3. Lastly, the lack of reactivity of mAb GH464 and 763.74 with peptides identified with mAb GH368, GH704, GH786 and GH1151 is associated with several differences in the amino acid sequences of their V_H and V_L-CDR3.

Example 3

Peptide mimics of the antigenic determinants recognized by anti-GD_3 ganglioside mAb.

Identification. Panning of phage display peptide libraries LX-8 and X_{15} with five anti-GD_3 ganglioside mAb resulted in the isolation of phage clones from the LX-8 library with mAb MG21 IgG1 and from the X_{15} library with mAb MB3.6 and MG22. No clones were identified following panning the two libraries with mAb MG21 IgG3 and R24.

Nucleotide sequence analysis of randomly selected positive clones identified one sequence from the clones identified with mAb MG21 IgG1 (referred to as clone #1), one sequence from those identified with mAb MG22 (referred to as clone #4) and two sequences from those identified with mAb MB3.6 (referred to as clones #2 and #3). The four amino acid sequences displayed no homology, except for the preferential use of Pro residues. This finding is in agreement with the identification of short consensus sequences, usually rich in aromatic amino acids and/or proline in carbohydrate mimicking peptides.

Clones #1 and #4 inhibited the binding of mAb MG21 IgG1 and MG22, respectively, to GD_3 ganglioside. The respective 50% inhibition concentrations were 1.3 and 12.3 M. In contrast clones #2 and #3 had only a marginal effect on the binding of mAb MB3.6 to GD_3 ganglioside.

The four clones reacted only with the mAb used for panning except for clone #1 which reacted also with mAb MG21 IgG3, although to a lower extent than with mAb MG21 IgG1.

Immunogenicity in BALB/c mice. Six out of the 14 BALB/c mice immunized with the phage displayed peptide identified with mAb MG22 developed anti-GD_3 ganglioside antibodies which crossreacted also with GD_2 and GM_3 gangliosides. These antibodies were inhibited in their reactivity with GD_3 ganglioside by a synthetic peptide corresponding to the phage displayed peptide identified with mAb MG22. The inhibition is specific, since the reactivity of the immune serum with GD_3 ganglioside was not affected by the

synthetic peptide #3 identified with mAb MB3.6. The kinetics of the development of the antibodies and their titers were heterogeneous in the six responding mice. Serum from mouse #3 reacted also with GD_3 ganglioside in a dot blot assay and displayed higher reactivity with GD_3 ganglioside expressing melanoma cells Melur than with GD_3 ganglioside negative lymphoid cells L14.

Example 4

Phage display peptide libraries. Phage display peptide libraries LX-8, XCX_{15} and X_{15} displaying disulfide constrained peptides XCX_8CX, single cysteine peptides XCX_{15} and random/linear peptides X_{15}, respectively, were kindly provided by Dr. J.K. Scott (Simon Fraser University, Burnaby, BC, Canada) (66). Methods of screening and panning phage display peptide libraries are well known in the art, for example, see US patents 5,763,164; 5,834,318; 5,733,731; 5,723,286; 5,565,325; 5,498,530; 5,750,373; and 5,807,986, all of which are herein incorporated by reference.

Peptide mimics of HMW-MAA are isolated by panning phage display peptide libraries with anti-HMW-MAA mAb 763.74 and with the eight HMW-MAA binding anti-anti-id mAb. The isolated phages are tested for reactivity in binding assays with the mAb used for panning. The amino acid sequence of the inserts of randomly selected phages among those found to be reactive with the mAb used for panning will be determined and utilized to synthesize peptides. The latter in turn are tested for their reactivity with mAb 763.74 and with the panel of HMW-MAA binding anti-anti-id mAb. Furthermore the sequence of the peptides is compared to the published amino acid sequence of HMW-MAA to determine the degree of homology.

Panning of phage display peptide libraries with anti-HMW-MAA mAb. Monoclonal antibodies (mAb) used for panning are purified from ascites by sequential caprylic acid and ammonium sulfate precipitation. Purity of the mAb is monitored by SDS-PAGE. Purified mAb are biotinylated using NHS-

LC-biotin (Pierce, Rockford, IL) according to the manufacturer's instructions. Micropanning of amplified phage display peptide libraries LX-8, XCX_{15} and X_{15} with biotinylated mAb is performed in 96-well microtiter plates (Falcon, Becton Dickinson, Lincoln Park, NJ) as described by Bonnycastle et al.. Briefly, the 1st round of panning is performed utilizing $1x10^{12}$ phage particles and 10g of biotinylated mAb per well. The subsequent three rounds of panning are carried out by decreasing the phage input to $1x10^{10}$ phage particles and the amount of biotinylated mAb to 0.10g per well. Eluted phages from each round of panning are amplified in *E. coli* K91kan prepared as described by Smith and Scott and used as an input for the next round of panning. Phage enrichment, i.e % yield, defined as the % of eluted phages / input phages, after each round of panning is determined by spot titering on NZY plates containing tetracycline (Tc) (20 ug/ml).

Reactivity of the isolated phage displayed peptides with the panel of anti-HMW-MAA mAb. The phage displayed peptides isolated from the recombinant peptide libraries following the fourth round of panning with anti-HMW-MAA mAb 763.74 and with the HMW-MAA binding anti-anti-id mAb are tested for specific reactivity with the mAb used for panning first by immunological screening and then by ELISA. In the immunological screening, random phage clones isolated from peptide libraries are tested. The ELISA is performed in flexible, U-bottom, 96 well microtiter plates (Dynatek, Chantilly, VA) which have been coated with the mAb used for panning by adding to each well 100ul of a mAb solution (100ug/ml of 0.05 M $NaHCO_3$, pH 9.6). Following an 18 h incubation at room temperature, wells are blocked with PBS containing 2 % BSA. Then, 100ul of a phage supernatant from overnight cultures of clones found to be positive in immunoscreening are added to each well. Following an overnight incubation at 4 C, biotinylated sheep anti-M13 antibodies (5 prime-3 prime Inc., Boulder, CO) are added and the incubation is continued for an additional 2 h at room temperature. Binding of biotinylated sheep anti-M13 antibodies is detected by incubating wells for 1 h at room temperature with a 1:2500 dilution of a SA-HRP solution. The reaction is developed using o-

phenylenediamine-H_2O_2 substrate and stopped with 2M H_2SO_4. Absorbance is read at 490 nm in an ELISA reader (EL 311, Bio-Tek Instruments Inc., Winooski, VT). Results are expressed as absorbance values read at 490 nm.

The specificity of the reaction is monitored by testing the reactivity of phage supernatants with an isotype matched mouse Ig. Clones found to be positive with the mAb used for panning are tested with the other anti-anti-id mAb.

Nucleotide sequence of phage inserts reacting with the mAb used for panning. Nucleotide sequence of peptide inserts from an average of 10 clones out of the clones found to react with the mAb used for panning, is determined by the dideoxy nucleotide chain termination method, with the following modifications. Purified phages are prepared from phage supernatant of individual clones by PEG/NaCl precipitation. Sequencing reactions are performed with $2x10^{11}$ phage particles in microtiter wells (GeNunc; Nunc, Roskilde, Denmark) using the SEQUENASE kit (version 2.0, United States Biochemical, Cleveland, OH) and ^{32}P-end-labeled f88.4 sequencing primer, 5'-CTGAAGAGAGTCAAAAGC-3'. The nucleotide sequences of the analyzed clones are translated into amino acid sequences. The amino acid sequences are compared to identify consensus sequences and the most frequently represented amino acid sequence. On the basis of this information, amino acid sequences are selected to synthesize peptides using standard 9-fluorenyl-methoxy-carbonyl (Fmoc) Solid Phase Peptide Synthesis (SPPS) in an automated peptide synthesizer (9050 Plus; Perceptive, MA). When indicated, synthetic peptides are cyclized using 5% dimethyl sulfoxide (DMSO) and purified by high-performance liquid chromatography (HPLC). Cyclization of peptides is confirmed by mass spectroscopy. Peptides are reconstituted in water at 5mM, aliquoted and stored at-20 C. If peptides are not soluble in water, they are dissolved in DMSO at 50mM and then diluted in the buffer to be used.

We Claim:

1. A method of treating disease in a mammal in need thereof comprising treating the mammal with an effective amount of a peptide mimic that elicits an immune response against a target molecule or a fragment thereof associated with the disease.

2. The method of claim 1 wherein the disease is cancer.

3. The method of claim 2 wherein the cancer is melanoma.

4. The method of claim 1 wherein the target molecule is a high-molecular weight melanoma associated antigen.

5. The method of claim 1 wherein the target molecule is a ganglioside gd3.

6. The method of claim 1 wherein the mammal is a human.

7. The method of claim 1 wherein the peptide mimic comprises the amino acid sequence SPSWYCPDCDKRPLV.

8. The method of claim 1 wherein the peptide mimic comprises the amino acid sequence RPYRYDPLGDLKSRH.

9. The method of claim 1 wherein the peptide mimic comprises the amino acid sequence EARNWHDFPIHPRTL.

10. The method of claim 1 wherein the peptide mimic comprises the amino acid sequence SCRWVGIDLYCP.

11. The method of claim 1 wherein the peptide mimic comprises the amino acid sequence EELHPPGSRAPSIRK.

12. The method of claim 1 wherein the peptide mimic comprises the amino acid sequence QCTGPNVATNCR.

13. The method of claim 1 wherein the peptide mimic comprises the amino acid sequence QCTGPNFATNCR.

14. The method of claim 1 wherein the peptide mimic comprises the amino acid sequence TCNGPSVYMNCL.

15. The method of claim 1 wherein the peptide mimic comprises the amino acid sequence RPYRYDPLGDLKSRH

16. The method of claim 1 wherein the peptide mimic is conjugated to an immunogenic compound.

17. The method of claim 1 wherein the peptide mimic is combined with an adjuvant.

18. The method of claim 1 wherein the peptide mimic is bound to MHC Class I Restricted Antigens forming a complex.

19. The method of claim 1 wherein the peptide mimic is bound to MHC Class II Restricted Antigens forming a complex.

20. The method of claim 1 wherein the peptide mimic is a synthetic peptide.

21. The method of claim 1 wherein the peptide mimic is a pseudopeptide.

22. The method of claim 1 wherein the peptide mimic is expressed on an antigen-presenting cell.

23. The method of claim 22 wherein the antigen-presenting cell is a dendritic cell.

24. A peptide mimic that elicits an immune response against a target molecule or a fragment thereof associated with a disease.

25. The peptide mimic of claim 24 wherein the disease is cancer.

26. The peptide mimic of claim 25 wherein the cancer is melanoma.

27. The peptide mimic of claim 24 wherein the target molecule is a high molecular weight melanoma associated antigen.

28. The peptide mimic of claim 24 wherein the target molecule is a ganglioside gd3.

29. The peptide mimic of claim 24 comprising the amino acid sequence SPSWYCPDCDKRPLV.

30. The peptide mimic of claim 24 comprising the amino acid sequence RPYRYDPLGDLKSRH.

31. The peptide mimic of claim 24 comprising the amino acid sequence EARNWHDFPIHPRTL.

32. The peptide mimic of claim 24 comprising the amino acid sequence SCRWVGIDLYCP.

33. The peptide mimic of claim 24 comprising the amino acid sequence EELHPPGSRAPSIRK.

34. The peptide mimic of claim 24 comprising the amino acid sequence QCTGPNVATNCR.

35. The peptide mimic of claim 24 comprising the amino acid sequence QCTGPNFATNCR.

36. The peptide mimic of claim 24 comprising the amino acid sequence TCNGPSVYMNCL.

37. The peptide mimic of claim 24 comprising the amino acid sequence RPYRYDPLGDLKSRH

38. The peptide mimic of claim 24 wherein the peptide mimic is conjugated to an immunogenic compound.

39. The peptide mimic of claim 24 wherein the peptide mimic is combined with an adjuvant.

40. The peptide mimic of claim 24 wherein the peptide mimic is bound to MHC Class I Restricted Antigens forming a complex.

41. The peptide mimic of claim 24 wherein the peptide mimic is bound to MHC Class II Restricted Antigens forming a complex.

42. The peptide mimic of claim 24 wherein the peptide mimic is a pseudopeptide.

43. The peptide mimic of claim 24 wherein the peptide mimic is expressed on an antigen-presenting cell.

44. The peptide mimic of claim 43 wherein the antigen-presenting cell is a dendritic cell.

45. The method of treating a disease in a mammal in need thereof comprising treating the mammal with an effective amount of a vector that

expresses a peptide mimic that elicits an immune response against a target molecule or fragment thereof associated with a disease.

46. The method of claim 45 wherein the disease is cancer.

47. The method of claim 46 wherein the cancer is melanoma.

48. The method of claim 45 wherein the target molecule is a high molecular weight melanoma associated antigen.

49. The method of claim 45 wherein the target molecule is a ganglioside gd3.

50. A vector that expresses a peptide mimic that elicits an immune response against a target molecule or fragment thereof associated with a disease.

51. The vector of claim 50 wherein the disease is cancer.

52. The vector of claim 51 wherein the cancer is melanoma.

53. The vector of claim 50 wherein the target molecule is a high molecular weight melanoma associated antigen.

54. The vector of claim 50 wherein the target molecule is a ganglioside gd3.

55. A nucleic acid molecule encoding a peptide mimic that elicits an immune response against a target molecule or fragment thereof associated with a disease.

56. The nucleic acid molecule of claim 55 wherein the disease is cancer.

57. The nucleic acid molecule of claim 56 wherein the cancer is melanoma.

58. The nucleic acid molecule of claim 55 wherein the target molecule is a high molecular weight melanoma associated antigen.

59. The nucleic acid molecule of claim 55 wherein the target molecule is a ganglioside gd3.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/27633

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A01N 37/18; A61K 38/00
US CL : 5142/13

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 5142/13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
search terms: melanoma, vaccine, antigen, peptide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,662,907 A (CYTEL CORPORATION) 02 September 1997, see entire document, especially col 1, lines 14-19, 30-35, 41-45, 58-60, col 2, lines 6-34, 50-55, col 4, 4-16, 35-37, 60-62, col 5, 14-31, col 7, 13-17.	1-4, 6, 16-21, 24-27, 38-42

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 18 MARCH 1999	Date of mailing of the international search report 02 APR 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SUSAN UNGAR Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/27633

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 7, 29
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 7 and 29 were not searched because they recite amino acid sequences of 16 consecutive amino acids without SEQ ID NO:s and therefore were not searchable

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-4, 6, 16-21, 24-27, 38-42

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/27633

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

Group IA, claims 1-4, 6, 7, 16-21, 24-27, 29 and 38-42, drawn to a method of treating a disease in a mammal comprising administering a peptide mimic that elicits an immune response against a target molecule associated with a disease and to the peptide mimic.
Group IB, claims 1-3, 5, 6 and 16-21 drawn to a method of treating a disease in a mammal comprising administering a peptide mimic that elicits an immune response against a target molecule associated with a disease.
Group IC, claims 1-4, 6, 8, 15 and 16-21 drawn to a method of treating a disease in a mammal comprising administering a peptide mimic that elicits an immune response against a target molecule associated with a disease.
Group ID, claims 1-4, 6, 9, and 16-21 drawn to a method of treating a disease in a mammal comprising administering a peptide mimic that elicits an immune response against a target molecule associated with a disease.
Group IE, claims 1-4, 6, 10, and 16-21 drawn to a method of treating a disease in a mammal comprising administering a peptide mimic that elicits an immune response against a target molecule associated with a disease.
Group IF, claims 1-4, 6, 11 and 16-21 drawn to a method of treating a disease in a mammal comprising administering a peptide mimic that elicits an immune response against a target molecule associated with a disease.
Group IG, claims 1-4, 6, 12 and 16-21 drawn to a method of treating a disease in a mammal comprising administering a peptide mimic that elicits an immune response against a target molecule associated with a disease.
Group IH, claims 1-4, 6, 13 and 16-21 drawn to a method of treating a disease in a mammal comprising administering a peptide mimic that elicits an immune response against a target molecule associated with a disease.
Group II, claims 1-4, 6, 14 and 16-21 drawn to a method of treating a disease in a mammal comprising administering a peptide mimic that elicits an immune response against a target molecule associated with a disease.

Group IIA, claims 24-26, 28 and 38-42 drawn to a peptide mimetic.
Group IIB, claims 24-26, 30,37 and 38-42 drawn to a peptide mimetic.
Group IIC, claims 24-26, 31 and 38-42 drawn to a peptide mimetic.
Group IID, claims 24-26, 32 and 38-42 drawn to a peptide mimetic.
Group IIE, claims 24-26, 33 and 38-42 drawn to a peptide mimetic.
Group IIF, claims 24-26, 34 and 38-42 drawn to a peptide mimetic.
Group IIG, claims 24-26, 35 and 38-42 drawn to a peptide mimetic.
Group IIH, claims 24-26, 36 and 38-42 drawn to a peptide mimetic.

Group IIIA, claims 45-48, 22 and 23 drawn to a method of treating a disease in a mammal comprising treating the mammal with an effective amount of a vector that expresses a peptide mimic of a high molecular weight melanoma antigen.
Group IIIB, claims 45-47, 49, 22 and 23 drawn to a method of treating a disease in a mammal comprising treating the mammal with an effective amount of a vector that expresses a peptide mimic of gD3.

Group IVA, claims 50-53,43 and 44 drawn to a vector that expresses a peptide mimic of a high molecular weight melanoma antigen.
Group IVB, claims 50-52, 54, 43 and 44 drawn to a vector that expresses a peptide mimic of gD3.

Group VA, claims 55-58 drawn to nucleic acid molecules encoding a peptide mimic for a high molecular weight melanoma antigen.
Group VB, claims 55-57 and 59 drawn to nucleic acid molecules encoding a peptide mimic for gD3.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The technical feature linking groups I-V appears to be that they all relate to a method of treating disease in a mammal comprising treating the mammal with a peptide mimic that elicits an immune response against a target molecule or fragment thereof

associated with the disease.

A special technical feature is a contribution over the prior art.

The invention of independent claim I is considered to lack a special technical feature because Chargelegue et al.(Peptide mimics of conformationally constrained protective epitopes of respiratory syncytial virus fusion protein.Immunology Letters.1997 Vol. 57 Nos 1-3, pages 15-17)teaches that a protective epitope of the conserved RSV fusion protein can be mimicked by synthetic peptides and that immunization with these mimotopes induced specific anti-RSV neutralizing antibodies and reduced viral load in vivo.

Therefore, the technical feature linking the inventions of Groups I-V does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

Technically the first recited group could consist solely of claim 1, however, in order to afford applicant a search most relevant to his invention the claims were defined as belonging to the groups set forth above. In each group the claim defining a particular species is in bold.

While the individual peptide species of the claims are related to either a high molecular weight melanoma antigen (7-14, 29-36) or gD3 (5, 28) they do not share a common core.